PROTOCOL FOR MIDIPREP OF MEDIUM YIELD PREPS: QIAGEN®

This protocol is adapted from Qiagen Qiafilter Midi Prep Protocol by the Gene Expression Lab.
This protocol is for use with Qiagen Plasmid Purification Systems. For additional technical inquiries, contact Technical Service at 800-DNA-PREP or www.qiagen.com

---

BEFORE STARTING THE EXPERIMENT

- Check for unwanted SDS precipitation in Buffer P2
- Pre-chill Buffer P3 to 4˚C

MIDIPREP PROTOCOL

1. Using a bacterial colony, inoculate a starter culture of ~3ml using LB broth containing the appropriate selective antibiotic. Incubate O/N at 37°C with shaking (225-300 rpm).
2. Using 100ul (medium yield) or 200ul (low yield) of the starter culture, inoculate a culture of 50 (medium yield) or 100ml (low yield) LB broth with the appropriate selective antibiotic. Grow O/N at 37°C with shaking (225-300 rpm).
3. Harvest the cells by centrifuging the culture at 3000rpm for 30 minutes at 4°C.
4. Resuspend the bacterial pellet in 5ml Buffer P1
   -If grow up 100ml culture due to low yield prep, increase buffers appropriately to make sure that lysis occurs efficiently.
5. Add 5ml of Buffer P2, mix gently but thoroughly by inverting 4-6 x's, and incubate at RT for 5min.
6. Add 5ml of chilled Buffer P3, mix immediately but gently by inverting 4-6 x's, and incubate on ice for 15 minutes.
7. Spin the lysate for ~5min at 3000RPM to clear the lysate slightly.
8. Add the cleared lysate to the Qiafilter Midi cartridge to filter further, incubating at RT for a few minutes before inserting plunger.
   - This allows any remaining precipitate to float to the top of the solution.

9. Equilibrate Qiagen-100 tip by applying 5ml of Buffer QBT (**mix resin WELL before applying to tip**), and allow the column to empty by gravity flow.
10. Apply the supernatant from step 6 to the Qiagen-tip and allow it to enter the resin by gravity flow.
11. Wash the tip w/ 2 x 10ml of Buffer QC.
12. Elute DNA w/ 5ml of Buffer QF.
13. Precipitate DNA by adding 3.5ml RT isopropanol to the eluted DNA. Mix and centrifuge @ 11000 rpm in a Sorval SS-34 rotor for 30minutes @ 4°C. Carefully decant the supernatant.
14. Dissolve the pellet in 400ul TE + 40ul 5M NaCl. Mix well, making sure that the pellet has dissolved in the TE and then transfer to a clean 1.5ml tube. Centrifuge for 5minutes at 4°C.
15. Transfer supernatant to a new tube and add 1ml of 100% ETOH.
16. Keep at -70°C for a minimum of 3hours or overnight.
17. Centrifuge for 20minutes at 13000rpm at 4°C. Carefully remove supernatant.
18. Wash with 1ml of 70% ETOH, centrifuge for 20 minutes at 13000rpm at 4°C, and carefully remove supernatant.
19. Solubilize in TE
    - 200ul for medium yields
    - 100ul for low yields
20. Use 1/20th of volume to test OD [10ul of medium yield to 1490ul TE (150dilution), and 5ul of low yield to 1495ul TE (300dilution)]

TROUBLESHOOTING

Little or no DNA after precipitation

- DNA did not precipitate – make sure that centrifuged for long enough (30 minutes) at a high enough speed.
- DNA pellet could have been lost as the isopropanol pellets are difficult to see due to their glassiness. It is helpful to mark the outside of the tube prior to spinning so that you know where the pellet is likely to be located.

Plasmid DNA was difficult to redissolve in TE

- There could be too much salt in the pellet. This is why we have incorporated the step where we do a wash with NaCl to remove excess salt.

Poor DNA yield

- It is possible that you lost the pellet when pouring off the isopropanol.

- The lysis step may not have been efficient. If cells are very dense or a larger volume of culture medium was used, the ratio of lysis to culture must be adjusted.